Biochemical, Morphological and Anatomical Changes in Tree Foliage Exposed to Vehicular-Pollution

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Abstract— In the quest of biomonitoring urban environmental health, the present study focuses on the assessment of tree foliage exposed to vehicular-pollution in Thane city, India. Tree species being continuously exposed to air pollution tends to absorb the pollutants by their foliage surface. Biochemical, morphological and anatomical changes in four selected tree species namely Alstonia scholaris, Cassia siamea, Ficus religiosa and Mangifera indica growing at Teen Haat Naka (polluted site) and Yeoor hills (forest area as control site) were investigated. It was observed that vehicular emissions strike air pollution tolerance index, leaf pigments (proline, carotenoids, phaeophytine), anticipated performance index, along with anatomical variations in stomata, palisade ratio and vein-islet ratio in selected tree species. The analysis revealed that for combating vehicular-pollution in urban areas Mangifera indica was found to be tolerant and excellent categorized tree species while Alstonia scholaris, Ficus religiosa and Cassia siamea as sensitive to vehicular exhaust. The variations in foliage architecture can serve as a biomonitoring tool of vehicular emissions in urban areas.

Keywords— biomonitoring vehicular pollution, ecosystem, environmental health, tree foliage, urban.

I. INTRODUCTION

Most of the urban areas of the globe have high concentrations of air pollutants emanating from various sources viz., traffic, motor vehicle, power generation, and residential areas located near industries (Lopez, et al., 2005). Air is the most vital asset for all living organisms for their healthy growth and development. However, highly developed urban areas are facing a severe air pollution issues raised by transportation through vehicular emissions. Several environmental factors like water, temperature, light, etc. affects on plant growth. Depending upon the level of uptake by plants, theses environmental factors may lead to abiotic stress. Abiotic stress in plants shows adverse effects on overall physiology, morphology and biochemical constituents of plants. Stress in plants considered as an evolutionary marker since few centuries. According to certain biotic and abiotic stress, plant responses are divided into two types, tolerance and avoidance. Tolerance in plants is physiological change in its metabolism depending on protoplast or allows stress to damage to be repaired, whereas avoidance is prevention of the stress in plants. As plants cannot move from one place to another, they have adopted a tendency to combat against pollutants (Larcher and Bauer, 1981).

Air pollutants, which are accumulated by plants, may be used as a bio-indicators of air pollution to highlight the effects for mapping their distribution in urban areas. Most of the developing countries have focused on quality of fuel content and vehicle parts. Although many of the old and outdated vehicles barrage on roads, with use of low grade fuel. Transportation associated with gasoline and burning of fuel in vehicles are considered as the highest source of air pollution, both at global and regional parts. Vehicular emission exhaust mainly consist of Sulphur dioxide (SO_2), carbon dioxide (CO_2), nitrogen oxide (NOx), and other volatile organic compounds with 60-70% particulate matter in the air (Prajapati and Tripathi 2008). The major pollutants emitted from diesel-fuelled vehicles are SO_2, NOx, particulate matter and polyaromatic hydrocarbons (PAH) while, from gasoline vehicles emits hydrocarbons (HC) and CO (Bhandarkar 2013).

Plants acts as a natural filters of air pollution either by stomatal uptake or the deposition on the surfaces of leaves. Air pollutant absorbed through stomata

undergoes various interactions and enhances the tolerance capacity of the plant to fight against the stress (Mittler 2002). All these interactions leads to different biochemical, physiological and anatomical responses in plants. Stress in plants can be evaluated by tracing some biochemical parameters, such as by monitoring air pollution tolerance index of plants.

In Thane, traffic intensity is high in many industrial, residential and commercial areas. The extent of vehicular air pollution levels in these regions yet not monitored. Such information however is necessary in controlling air pollution in urban areas to provide guideline studies on the air pollution in various metropolises cities worldwide. Furthermore, knowledge of the plants that is able to tolerate vehicular air pollution and act as a sink for the toxic gases would be biomonitoring eco-friendly tool in controlling air pollution along major roads with heavy traffic and higher intensity of vehicular emissions. The outcome from this research will contribute for controlling air pollution in fast growing urban metropolis, as well as it will serve as an important source of information of tolerant plants species.

II. MATERIAL AND METHOD

2.1 Study area

Thane city is located in Maharashtra state and is a part of the Mumbai metropolitan region. Thane city lies between 19° 12' N and 73° 02' E with the total area of 128.23 sq.km. The maximum temperature ranges from 35°C to 40°C during summer and the minimum temperature is between 25°C to 35°C during the winter months of November-January. The average rainfall is about 2500 mm received during the rainy season from June to the end of September. The climate of the region is coastal, hot and humid. Location of the study area and metrological data for the year 2016 presented in the following figure 1.

2.2 Selection of sites for analysis of other parameters

Two sites, one control (Yeoor hill) & one polluted (National highway from Marathon chowk (popularly known as Teen Haat Naka) to Ghodbander road) was selected for the determination of Enzymatic parameters, Dust fall on leaves, Anticipated performance index (API), Stomatal index, Vein-islet number, Palisade ratio, Phenol, Carotenoids and Phaeophytin.
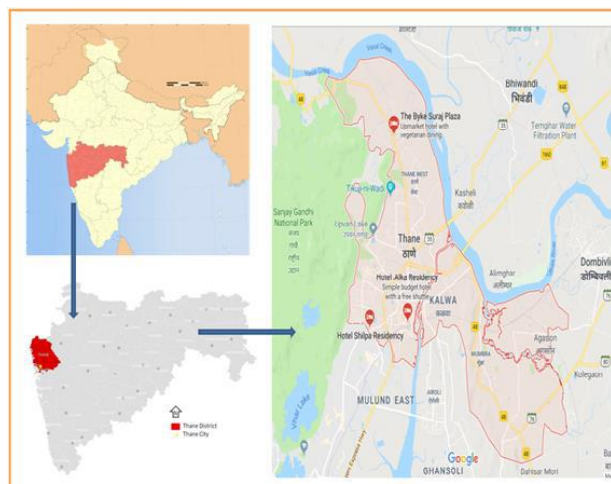

Fig 1: Location of India, Maharashtra, Thane District and Thane city

2.3 Polluted site

National highway-48 from Marathon chowk (popularly known as Teen Haat Naka) to Kasarvadavali on Ghodbunder road, Thane, Maharashtra, which is the busiest highway is selected as polluted site. This is an express highway connects to Agra and further to national capital Delhi. Also, there is the greater intensity of dust accumulation on highway roads. Traffic density and anthropogenic activities carried out mainly on the road. Four-wheeler emission and transportation of heavy-duty vehicles observed greater as compared to other roadways. Figure 2 shows the location of selected polluted site on the map.
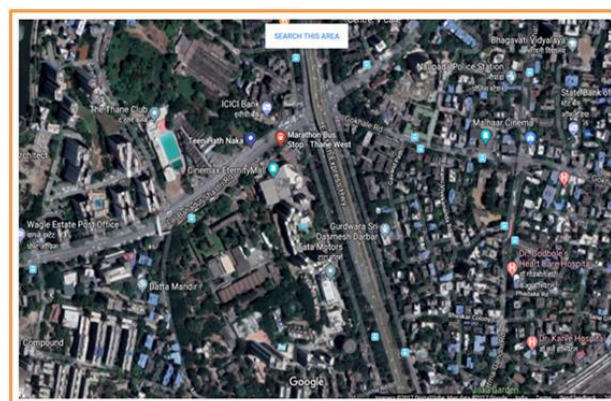

Fig 2: Teen Haat Naka (polluted site)

2.4 Sampling

All plant species taken up for the dust, heavy metal and other parameters analysis were at height of 2, 3 m from ground level with high amount of dust particles on the foliage surface. Foliage facing towards roadside was collected early in the morning from 8 am to 10 am by

random selection. All plant foliage collected in separate labeled zip locker polythene bags. Care was taken while carrying it to the laboratory for further analysis.

2.5 Estimation of total chlorophyll

One gram of the greenest leaves of the plants were selected and cleaned thoroughly with water and dried in room temperature for a while. Then leaf samples macerated in a pestle with mortar adding 20 – 25 ml of 80% acetone. A pinch of magnesium carbonate was added to the leaf material while grinding. The content was centrifuged at 2000 r.p.m. for 15 minutes in cold centrifuge. Transferred the extract to a volumetric flask and made to the volume of 50 ml. using 80% acetone. Optical density of this green solution was read at 645 nm (D645) and 663 nm (D663) using spectrophotometer and the total chlorophyll was calculated with the following formula by Arnon, 1949.

Total Chlorophyll = 20.2 (D645) + 8.02 (D663)   (1)

2.6 Measurement of leaf extract pH

pH is the measure of hydrogen ion activity and mostly depends on the relative amounts of the adsorbed hydrogen and metallic ions. It is a good measure of the intensity of acidity and alkalinity of suspension. Five gram of the fresh leaves was homogenized in 10 ml. demonized water. This was filtered and the pH of the leaf extract determined after calibrating pH meter with buffer solution of pH 4, 7 and 9.

2.7 Estimation of relative water content (RWC)

Fresh weight was obtained by weighing the fresh leaves. The leaves were then immersed overnight in water blotted, dry and then weighed to get the turgid weight. The leaves were then dried overnight in an oven at 70°C and reweighed to obtain the dry weight. RWC was determined and calculated by following formula by Barr and Weatherley, 1962.

RWC (%) = [(FW-DW)] / [(TW-DW)] x 100   (2)

Where,

FW is Fresh weight,

DW is Dry weight,

TW is Turgid weight

2.8 Estimation of ascorbic acid (AA)

One gram of ground fresh leaves was homogenized in 4 ml oxalic acid - EDTA extracting solution for 30 seconds. 1 ml. of orthophosphate acid and 1 ml 5% tetraoxosulphate (vi) acid were added. 2 ml. of ammonium molybdate and 3 ml. of water were added. The solution was left to stand for 15 min. The absorbance was

read off with a digital spectrophotometer at 760 nm. The concentration of the ascorbic acid was determined from a standard ascorbic acid regression curve by Bajaj and Kaur, 1981.

2.9 Air pollution tolerance index (APTI) determination

The air pollution tolerance indices of ten common plants were determined by the following method by Singh and Rao, 1983.

APTI = [A x (T+P) + R] / [10]   (3)

Where,

A is Ascorbic acid (mg/g. fr.wt),

T is Total chlorophyll (mg/g. fr.wt),

P is Leaf extracts pH and

R is Relative Water Content

The APTI values help to identify the sensitive species to be used for assessing the air pollution tolerant index of plants as to determine the air pollution tolerant species.

2.10 Anticipated performance index (API)

Table 1: Gradation of plant species based on APTI and other biological and socio-economic characters

Sr. No.	Grading	Character	Pattern of assessment	Grade allotted
A	Tolerance	Air pollution tolerance index (APTI)	12.0-16.0	+
			16.1-20.0	++
			20.1-24.0	+++
			24.1-28.0	++++
			28.1-32.0	+++++
			32.1-36.0	++++++
B	Biological & Socio-Economic	(i) Plant habitat	Small	-
			Medium	+
			Large	++
		(ii) Canopy structure	Sparse/irregular globular	-
			Spreading crown/ open semi dense	+
			Spreading dense	++
		(iii) Type of plant	Deciduous	-
			Evergreen	+
		(iv) Laminar structure : size	Small	-
			Medium	+
			Large	++
		: Texture	Smooth	-
			Coriaceous	+
		: Hardiness	Delineate	-
			Hardy	+
		(v) Economic value	Less than three uses	-
			Three or four uses	+
			Five or more uses	++

By combining the resultant of APTI values with some relevant biological and socio-economic characters (plant habit, canopy structure, type of plant, laminar structure &

economic values), the API was evaluated for different species. Different grades (+ or -) are allotted to plants depending upon the characters. Plants are scored according to their grades (Mondal et al. 2011). The criteria used for calculating the API of different plant species are given in Table 1 and Table 2.

Table 2: Anticipated Performance Index (API) of plant species

Grade	Score (%)	Assessment category
0	Up to 30	Not recommended
1	31-40	Very poor
2	41-50	Poor
3	51-60	Moderate
4	61-70	Good
5	71-80	Very good
6	81-90	Excellent
7	91-100	Best

2.11 Stomatal index

Stomatal index evaluated as it is the percentage of numbers of stomata produced to the total number of epidermal cells. Every stoma counted as one cell. The stomatal number varies considerably with the age of the leaf, and due to changes in environmental conditions, the stomatal index is relatively constant and, therefore, of diagnostic significance for a given species. Same leaf preparations were used as for stomatal number. Some epidermal cells and stomata (the two guard cells and ostiole considered as one unit) were counted within the square (Evans, 2002; Kokate, 2006). Observed under 10X (eyepiece) and 40X (objective). Stomatal index evaluated by using the following equation.

$$\text{Stomatal index (S.I.)} = (S/E+S) \times 100 \qquad (4)$$

Where,

S = number of stomata per unit area

E = number of epidermal cells in the same unit area

2.12 Vein-islet number

The vein-islet is the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. Vein-islet number is defined as the number of vein-islets per sq mm of the leaf surface, midway between the midrib and the margin. The leaf sample, after soaking in water, was treated successively with sodium hypochlorite to bleach, 10% hydrochloric acid to remove Ca oxalate and finally chloral hydrate. The camera lucida was set up and by means of a stage micrometer the paper

was divided into squares of 1 sq mm. In the cleared preparation veins were traced in four continuous squares, in a square of 2 mm×2 mm. Each vein-islet was numbered during counting (Evans, 2002). The range and average was determined in 10 sets of 2 mm×2 mm area (Kokate, 2006). Observed under 10X (eye piece) and 4X (objective).

2.13 Palisade ratio

Palisade ratio is defined as the average number of palisade cells beneath each epidermal cell. Pieces of leaf about 2 mm square were cleared by boiling with chloral hydrate solution. First a number of groups each of four epidermal cells were traced and their outlines made more conspicuous. The palisade cells lying beneath each group were then focused and traced. The palisade cells in each group were counted, cells which were more than half covered by the epidermal cells were also counted; the figure obtained was divided by 4 to obtain palisade ratio of that group (Evans, 2002). Twenty five groups from different leaf samples were determined for the calculation of range and average (Kokate, 2006). Observed under 10X (eye piece) and 40X (objective).

2.14 Determination of Proline

Determination of proline in leaf samples (replicates) was done by (Bates et al. 1973) method. Plant Dry matter (0.05 g) homogenized in 5 ml of 3% aqueous sulfosalicylic acid. Leave the same for 3hrs for complete extraction. Centrifuged the sample at 1500 g for l0 min. After that 2 mL of supernatant was added to 2mL Glacial Acetic acid and 2 mL Acidic Ninhydrin. (Warmed1.25 g ninhydrin in 30 ml of GAA + 20 ml (6M) H_3PO_4 with agitation until dissolved) Boiled at 100°C in a Water Bath for 60 min. Stopped the reaction by placing in an ice path. Then added 4 ml of Toluene and mix vigorously. Mixuture was allowed to warm at room temperature. The optical density was read at 520 nm, using toluene as blank. At 4 °C, the reagent was made stable for 24 h. The standard curve was used for concentration from 0- 512 µL (20-100 µg/ml) of proline.

2.15 Measurement of chlorophyll, carotenoids and pheophytin pigments

The chlorophyll pigments in the leaves estimated following the method of Arnon (1949). The fully expanded leaves from all the sites were collected in the polythene bags and transported to the laboratory. The leaves were washed out thoroughly with distilled water. Three replicates were used for each plant. Weighted fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of the acetone extract was

made up to a known one, and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer. The concentration of the chlorophyll pigments was calculated using the following formula, and the results are expressed in mg/g fresh weight.

Chlorophyll (mg/gm fresh weight):

Chlorophyll a = [(12.7 X OD at 663) – (2.69 X OD at 645)] X dilution factor   (5)

Chlorophyll b = [(22.9 X OD at 645) – (4.68 X OD at 663)] X dilution factor   (6)

Total chlorophyll = [20.2 X OD at 645) – (8.02 X OD at 663)] X dilution factor   (7)

Carotenoids (mg/gm fresh weight):

Carotenoids = [(7.6 X OD at 480) – (1.49 X OD at 510)] X dilution factor   (8)

Phaeophytin (mg/gm fresh weight):

Phaeophytin = [(6.75 X OD at 666) + (26.03 X OD at 655)] X dilution factor   (9)

III. RESULTS

3.1 Variation in total chlorophyll

The total chlorophyll content of all the four tree species at the Teen Haat Naka road site was lower and significantly different than those of the control site. The concentration of total chlorophyll ranged from 3.36 mg/g in *Ficus religiosa* at Teen Haat Naka road site to 2.35 mg/g in Alstonia scholaris. While, at the control site, total chlorophyll content range from 4.10 mg/g in *Ficus religiosa* to 3.3 mg/g in *Mangifera indica* (Table 3).

Table 3: Air pollution tolerance index (APTI) of trees at Teen Haat Naka road site

Scientific name of tree species	Total chlorophyll		pH of leaf extract		Relative water content		Ascorbic acid		APTI	
	mg/gm				%		mg/gm			
	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted
Alstonia scholaris	4.4 ± 0.13	2.35 ± 0.06	6.0 ± 0.0	5.78 ± 0.04	92.2 ± 3.48	86.0 ± 1.00	3.7 ± 0.10	3.42 ± 0.01	13.06 ± 0.22	11.37 ± 0.10
Cassia siamea	4.60 ± 0.10	3.12 ± 0.11	5.80 ± 0.0	6.58 ± 0.00	55.3 ± 1.10	54.3 ± 1.78	3.60 ± 0.10	5.03 ± 0.06	9.277 ± 0.22	10.31 ± 0.19
Ficus religiosa	4.10 ± 0.27	3.36 ± 0.04	6.10 ± 0.0	7.36 ± 0.00	56.70 ± 1.54	51.19 ± 1.06	5.30 ± 0.10	2.9 ± 0.02	11.07 ± 0.28	8.225 ± 0.12
Mangifera indica	3.3 ± 0.10	2.70 ± 0.01	6.2 ± 0.0	6.29 ± 0.00	92.9 ± 6.78	70.14 ± 2.16	7.9 ± 0.66	5.69 ± 0.19	16.79 ± 0.25	12.15 ± 0.35

* Mean ± SEM for triplicates of trees showing the values for the biochemical parameters

3.2 Variation in Leaf extract pH

The ph of the leaf plays an important role in balancing the acidic and basic nature. It is also involved in many enzymatic and protein formation reaction. The plants showed a variation in leaf extract pH among the study sites. Higher pH was recorded for *Ficus religiosa* while, lower in *Alstonia scholaris* (Table 4).

3.3 Variation in relative water content (RWC)

Relative water content of leaf samples of all the four tree species at the Teen Haat Naka road site was higher but not significantly different from those at the control site (Table 3). Relative water content of leaf samples at Teen Haat Naka site ranged between 68.38 and 93.86%, while those at the control site were ranging from 51.19 to 86.0 %. *Alstonia scholaris*, *Mangifera indica*,*Cassia siamea* had higher relative water content at the Teen Haat Naka road sites than at the control site.

3.4 Variation in Ascorbic acid content

The ascorbic acid content of all the four tree species at the Teen Haat Naka road site was higher and significantly different than those of the control site (p = 0.000). The mean concentration of ascorbic acid ranged from 2.09 mg/g in *Ficus religiosa* to 5.69 mg/g in *Mangifera indica* at Teen Haat Naka road site. While at the control site, ascorbic acid content range from 7.9 to 3.60 mg/g in *Mangifera indica* and *Cassia siamea* (Table 3).

3.5 Variation in air pollution tolerance index

The air pollution tolerance index calculated by ascorbic acid content, pH of the leaf extract, total chlorophyll content and the relative water content were used in evaluation of the sensitivity level to the vehicular exhaust (Table 3). The variation in air pollution tolerance index was recorded in range of 12.15 to 8.22 in *Mangifera indica* and *Ficus religiosa* at the Teen Haat Naka road site. While at control site, it was ranged higher in the same

plants between 16.79 to 9.27. The Pearson correlation coefficient revealed the relationship between the four biochemical parameters with a dependant parameter APTI (Table 4).

Table 4: Correlation coefficient matrix

	APTI	API grade	Proline	Careotenoid	Stomatal Index	Palisade Ratio	Vein-islet number	Pheaeophytin
APTI	1.000							
API grade	0.441	1.000						
Proline	0.864	0.602	1.000					
Careotenoid	-0.125	0.081	-0.501	1.000				
Stomatal Index	-0.548	0.458	-0.149	-0.108	1.000			
Palisade Ratio	-0.288	0.480	0.215	-0.526	0.890	1.000		
Vein-islet number	-0.548	0.458	-0.149	-0.108	1.000	0.890	1.000	
Pheaeophytin	-0.789	-0.720	-0.983*	0.480	-0.028	-0.361	-0.028	1.000

*significant at 0.01%

3.6 Evaluation of Anticipated Performance Index

The tree species with excellent grades were categorized as per the grading pattern and anticipated index scores (Table 1 & 2). The excellent and very good category trees were proposed for the development of greenbelt in urban areas. According to the garding pattern and score of the tree species *Mangifera indica* assessed as an excellent with score (81.3), while *Cassia siamea* (68.8) as a good categorized tree species (Table 5).

Table 5: Anticipated Performance Index (API) of plant species

Grading Character	Tree Species			
	Alstonia scholaris	*Cassia siamea*	*Ficus religiosa*	*Mangifera indica*
APTI	+	+	+	+
Tree habitat	+ +	+ +	+ +	+ +
Canopy structure	+ +	+ +	+ +	+ +
Tree type	+	+	+	+ +
Leaf size	+ +	+	+ +	+ +
Texture	+	+	+	+
Hardiness	+	+	+	+
Economic importance	+ +	+ +	+ +	+ +
Total plus	12	11	12	13
Grade allotted % Scoring	75	68.8	75	81.3
API grade	5	4	5	6
Assessment	V. Good	Good	V. Good	Excellent

3.7 Variation in Carotenoid

The carotenoid content of leaf samples of all the four tree species at the Teen Haat Naka road site was lower than and significantly differed from those at the control site. The mean concentration of carotenoid ranged from 0.55 mg/g in *Alstonia scholaris* to 1.19 mg/g in *Mangifera indica* Teen Haat Naka road site. While, at the control site, carotenoid content ranged from 1.11 mg/g in *Ficus religiosa* to 1.28 mg/g in *Cassia siamea* (Figure 3)
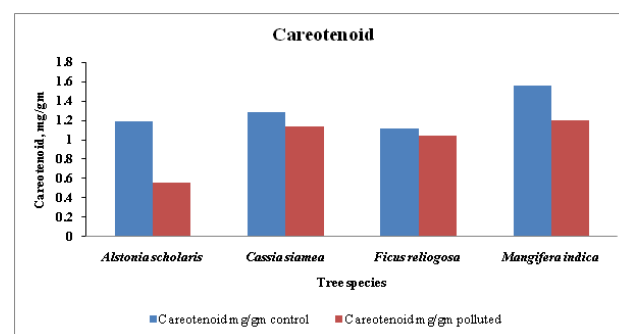

Fig 3: Carotenoid content in plants at control and polluted (TeenHaat Naka Raod) site

3.8 Variation in Phaeophytin content

The phaeophytin content of all the four tree species at the Teen Haat Naka road site was higher and significantly different than those of the control site (p = 0.000). The mean concentration of phaeophytin content ranged from 6.25 mg/g in *Cassia siamea* to 3.73 mg/g in Alstonia scholaris. While at the control site, phaeophytin content range from 7.06 to 6.35 mg/g in *Cassia siamea* and *Ficus religiosa* (Figure 4).
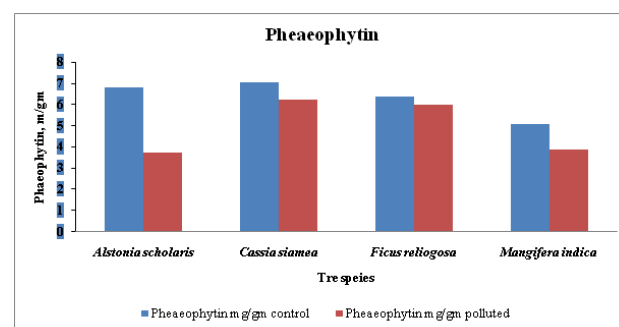

Fig 4: Phaeophyin content in plants at control and polluted (TeenHaat Naka Raod) site

3.9 Variation in Proline content

The proline content of leaf samples of all the four tree species at the Teen Haat Naka road site was lower than and significantly differed from those at the control site. The mean concentration of carotenoid ranged from 16.32 mg/g in *Ficus religiosa* to 24.8 mg/g in Alstonia scholaris. While, at the control site, proline content ranged from 4.1 mg/g in *Cassia siamea* to 5.2 mg/g in *Alstonia scholaris* (Figure 5).
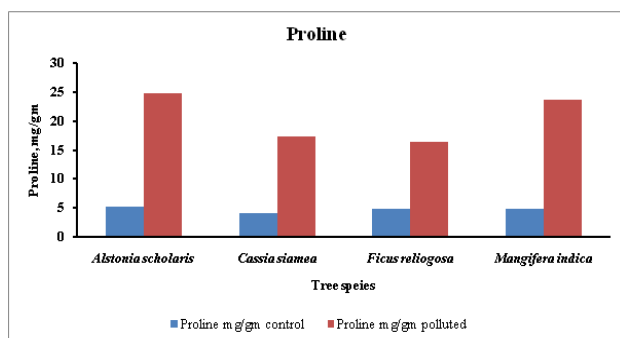

Fig 5: Proline content in plants at control and polluted (TeenHaat Naka Raod) site

3.10 Variation in Stomatal index

The stomatal index of all the four tree species at the Teen Haat Naka road sites were higher and significantly different than those of the control site (p = 0.000). The mean concentration of stomatal index ranged from 22.73 in *Ficus religiosa* to 13.33 mg/g in Cassia siamea. While at the control site, stomatal index range from 23.81 to 15.63 mg/g in *Alstonia scholaris* and *Cassia siamea* (Figure 6).
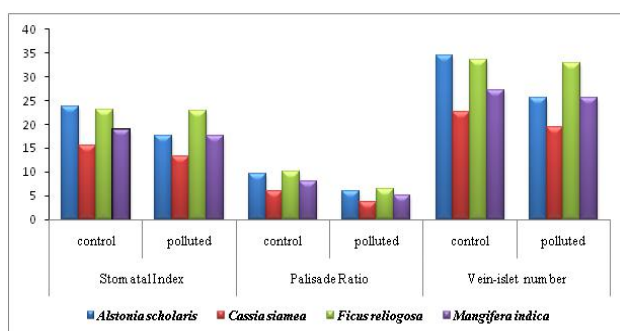

Fig. 6 : Stomatal index, palisade ratio and vein islet ration of plants at control and polluted (Teen Haat Naka Raod) site

3.11 Variation in Palisade ratio

The palisade ratio of leaf samples of all the four tree species at the Teen Haat Naka road site was lower than and significantly differed from those at the control site

(Figure 6). The mean concentration of carotenoid ranged from 6.35 in *Ficus religiosa* to 3.76 mg/g in *Cassia siamea*. While, at the control site, palisade ratio ranged from 10.03 in *Ficus religiosa* to 5.93 in *Cassia siamea.*

3.12 Variation in Vein-islet number

The vein-islet number of all the four tree species at the Teen Haat Naka road site was higher and significantly different from those of the control site (p = 0.000). The mean concentration of vein-islet number ranged from 22.73 in *Ficus religiosa* to 13.33 mg/g in *Cassia siamea*. While at the control site, vein-islet number range from 23.81 to 15.63 mg/g in *Alstonia scholaris* and *Cassia siamea* (Figure 6).

3.13 Association between biochemical, physiological and morphological parameters

The association between the biochemical, physiological and morphological parameters of four tree species *Ficus religiosa, Cassia siamea*, *Alstonia scholaris* and *Mangifera indica* was analysed with Pearson correlation coefficient. The analysis revealed a significant relationship between the determined air pollution tolerance index of tress as independent variable with physiological parameters (API), anatomical characters (stomatal index, palisade ratio, vein islet ratio) and the other leaf pigments(proline, carotenoid, and phaeophytin) as variable parameters. The Pearson correlation revealed that APTI is positively correlated with Proline and anticipated performance index grade while, proline was negatively correlated with carotenoid, stomata and vein-islet ratio. A negative correlation of APTI was recorded with carotenoids, stomatal index palisade ratio and Vein-islet number while, carotenoid is negatively correlated with stomata, vein and palisade (Table 4)

IV. DISCUSSION

In this study the chlorophyll content was observed higher in *Ficus religiosa* while, lower in *Alstonia scholaris* at selected study site than control site. Similar findings were recorded by Raza and Murthy,1988 that chlorophyll content in plants is dependent on the scale of pollutant absorbance by roots, leaves. Giri et al., 2013 recorded that effect of air pollutants emitted from the exhaust of automobiles and industries on the chlorophyll content of leaves. Higher pH was recorded for *Ficus religiosa* while, lower in *Alstonia scholaris*. Similar observations were recorded by Singare and Talpade (2013) who concluded that the leaf extract pH response to air pollution with a decrease in pH content when compared to control site. Some authors reported that in polluted sites trees show a

higher level of pH, which is responsible for the tolerance level of acidic air pollutants (Singh et al., 1991). High pH helps in the conversion of hexose sugar into ascorbic acid effectively by producing reactive oxygen species (ROS).

Alstonia scholaris, *Mangifera indica*,*Cassia siamea* had higher relative water content at the Teen Haat Naka road sites than at the control site. The large quantity of water (RWC) in tree helps in maintaining its physiological balance under stress conditions of pollution. Maximum moisture content favors drought resistance in trees. Water is the crucial requirement for plant life, as the decreased water content may cause severe stress conditions (Singh and Verma, 2007). Higher level of water content will help the trees to maintain its biochemical as well as physiological balance in highly polluted areas (Seyyednejad et al., 2011, Chandawat, et al., 2011).

The concentration of ascorbic acid varied in *Ficus religiosa* to *Mangifera indica* at Teen Haat Naka road sites. Ascorbic acid plays a significant role in resistance to air pollution as it acts as an antioxidant was found in growing parts of trees (Keller and Schwager, 1977; Pathak et al., 2011). The increased concentration of ascorbic acid in trees helps to fight against the pollutants stress (Chattopadhyay et al., 2010). The plants growing at industrial sites showed an increased amount of ascorbic acid content. The results goes in line with findings of Radhapriya et al., 2012 that plants growing near cement industry exhibited higher amount of ascorbic acid.

All plant species showed a tremendous variation and difference in all studied parameters. The air pollution tolerance index of the plants decides its tolerance and sensitivity towards air pollution. The variation in air pollution tolerance index was recorded in range of 12.15 to 8.22 in *Mangifera indica* and *Ficus religiosa* at the Teen Haat Naka road site. The results goes in hand with hand with the outcome of an assessment for evaluating the air pollution tolerance index and air pollution performance index of some plants growing nearby Neyveli thermal power plant by (Govindaraju, et al., 2012; Seyyednjad, et al., 2011).

In urban forests, it is necessary to find out some of the tolerant plant species for developing a green belt. APTI along with the API acts as an excellent tool for the better understanding of plants. Similar findings were recorded by (Mondal et al., 2011; Eshfahani et al.2015), who analyzed the API of ten tree species from an urban forest for an important biological, economic parameters and APTI.

It was observed that plants growing alongside of highway and polluted areas showed decreased stomatal index as compared to control site. The current analysis of stomatal index revealed that the adaxial surface of the leaves of selected plant species was found to vary among the species growing at different study locations. Similar observations were carried out by (Bermadinger et al., 1988), reported that certain particulates when reacts with epidermal cells release some toxic substances. These toxic substances accelerate the rate of photosynthesis and remove the cuticular wax of leaves. Accumulation of dust blocks the stomata and thus results in decreased rate of photosynthesis and transpiration. The results are in line with (Lincoln et al., 2006), reported that dust on leaves responsible for blockage of stomata thus shows visible changes in leaf anatomy, physiology, and other biochemical parameters like chlorophyll, carotetenoids and other enzymatic activities. The results revealed that effect of air pollution decreases the vein islet number in plants.

The vein-islet ratio was found to be changing with different environmental conditions. Similar observations were carried out by (Steubing, et al., 1989) dust deposition and certain atmospheric gaseous pollutants recorded as the major responsible factors for leaf damages, limited plant growth, necrosis, seed germination, flowering and many biochemical and physiological changes. Palisade ratio was seen affected by emission of pollutants resulting in decreased in ratio when compared with control site plants. The current study revealed that increased pollution levels decreased the palisade ratio in plants. Singh et al.2002, carried out similar findings, with study on stomatal conductance, palisade ratios and recorded that deposition of dust leaf surface shows adverse effect on optical density of leaf.

Present analysis of proline in selected plants revealed that it was higher in all selected plants than control site. The results are in agreement with Verbruggen et al., 2008, who reported that proline could be used for selecting stress tolerant species. It is concluded from the current research that plants respond to air pollution according to their sensitivity and stress tolerance capacity. (Jaleel et al., 2007, Yancy et al., 1982, Ozturk and Demir, 2002) reported similar conclusion. Increased amount of proline in plants is considered for increased stress in plants.

It was observed from the study that carotenoid content in leaves all selected plant species recorded a decreased carotenoid content when compared with control site. Content of caroteniods varies according to species as well as climatc conditions. Goldsmith et al., 1976, observed that at polluted sites the plants showed early senescence, which was due to, changed structure of carotenoid pigment. Findings of the study suggest that plants showed a decreased level of carotenoids at polluted site. The results are in line with Bhattacharjee et al., 1994;

Sinha et al., 2002, that high pollution was the responsible factor for low carotenoid content and thus overall growth and development of plants.

The phaeophytin content in plants at polluted site observed to decreased when comapared to polluted site. Dollard et al., 1986 concluded in his research that air pollutants exposed plants showed a very low rate of photosynthesis. Singh et al., 1997, Khan et al., 1990, concluded similar findings that decrease in chlorophyll-a molecule was due to explosion to SO_2, which eventually showed the low rate of chlorophyll content in leaves. The main reason behind transformation of chlorophyll into phaoephytin molecule is as SO_2 dissolved in water matrix present on the cell surface and thus reacts with cell molecules

V.   CONCLUSION

The results of the present study provide information that the vehicular pollution of the Thane city is creating trouble not for all organisms but also for the plants. Plants in urban areas are continuously exposed to air pollutants, ensuing accumulation of pollutants and their integration into their system, resulting in changing the nature of leaf and its tolerance and sensitivity. This sensitivity is measured through various biochemical parameters and finally through APTI. Since this, determination of APTI is more appropriate than before. The plant that shows higher index value is tolerant to air pollution and can be used as a sink to control pollution. The plants with lower index value seemed to be sensitive and used as bioindicator to recognize levels of air pollution. Thus, trees can utilize as tolerant or sensitive towards air pollution. Air pollution in the urban region is on the rise and augmented significantly due to increased vehicular pollution, urbanization and fast increase in small-scale industries.

VI.   ACKNOWLEDGEMENT

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